Pharmacodynamic Modulation of Motility by Glycine Administration in Dugesia Dorotocephala

BOGDAN HORATIU SERB[1], ALINA ORMENISAN[2]*, ANCUTA PLESERU[1], VLAD PRELUCA[1], MIHAELA CERNUSCA MITARIU[1], CORINA ROMAN FILIP[1], HORIA MIHAIL BARBU[3], SEBASTIAN CERNUSCA MITARIU[1]

[1] Lucian Blaga University of Sibiu, Faculty of Medicine, 2A Lucian Blaga Str., 550169, Sibiu, Romania

[2] University of Medicine and Pharmacy Targu Mures, Faculty of Dental Medicine, 38 Gh. Marinescu Str., 540139, Targu Mures, Romania

[3] Titu Maiorescu University of Bucharest, Faculty of Dental Medicine, 67A Gh. Petrascu Str., 031593, Bucharest, Romania

The glycinergic neurotransmission system is a potential pharmacological target for pain and muscle spasm relief therapy. Planarians are attractive model organisms for neuropharmacological research, due to their simplicity and neurotransmitters shared with mammals. In this study, planarians were exposed to glycine, caffeine, midazolam and ondansetron separately and to successive associations of glycine/caffeine, glycine/midazolam and glycine/ondansetron. Effects on motility were examined by a grid crossing assay. Results showed a dose-dependent reduction of motility by glycine, partially reversed by caffeine and midazolam, but not by ondansetron. Midazolam caused an intense decrease of motility, but not significantly lower than glycine. Ondansetron-treated planarians showed decrease motility compared with controls, but not with glycine or midazolam. These results are consistent with data obtained from higher organisms, showing that planarians can be used as model organisms for glycinergic pharmacological research.

Keywords: glycine, motility, planarians

The glycinergic neurotransmission system is involved in multiple neurologic functions, including motor and sensitive inhibitory signaling, sensory signal processing, spinal reflex coordination and neural development. Of special interest is its role in nociceptive integration, as a potential target for pain relief medication [1].

Planarian flatworms of the Dugesia genus have been classically used as model organisms for regeneration and spatial specification signaling [2]. However, they possess several traits that recommend them for neuropharmacological research. Their small size and ease of husbandry makes planarian research very accessible from an economic perspective. Because they are the simplest organisms that present cephalization of the nervous system [3] and a wide array of neurotransmitters in common with mammals [4], planarians offer the fundamental pharmacological targets without the confounding variables present in more complex organisms.

While classical neurotransmitters and their targets in planarians have been relatively well explored [5], there is very little research regarding the planarian glycinergic system.

Experimental part

Dugesia dorotocephala planarians measuring 8.5-9 mm in length were obtained from Carolina Biological Supply (Burlington, North Carolina, USA) and maintained at 21°C in standardized solution ($HCO3^-$ 339.5 mg/L, Ca^{++} 58.34 mg/L, Mg^{++} 32 mg/L, Na^+ 2.5 mg/L; *p*H 7.43).

Glycine was purchased from Sigma Aldrich Chemical Company (Steinheim, Germany) and used to prepare 10, 50 and 100 ìM solutions. Caffeine was purchased from Loba Chemie Company (Viena, Austria) and used to prepare a 50 μM solution, dose that had shown to be effective in the previous articles [4]. Midazolam (Dormicum, Roche Romania) and ondansetron (Osetron, Dr Reddy's Laboratories Romania) used in dilutions of 20 and 10 μM, respectively.

Solutions were freshly prepared in the day of the experiments.

Seven groups (n=7) of planarians were incubated in Petri dishes with 10 mL of glycine, caffeine, midazolam and ondansetron solutions in the aforementioned concentration for 60 min, followed by transfer in a 9 cm Petri dish marked with gridlines 1 cm apart, containing 20 mL of water. The planarian activity was recorded for 5 min, counting the number of gridlines crossed, expressed as average crosses/min.

Subsequently we tested associations of glycine (100 μM) with the follow substances: caffeine (50μM), midazolam (20μM) and ondansetron (10μM). The planarians were incubated with 10 mL of 100 μM glycine for 60 min, followed by transfer and incubation for 60 min with 10 mL of the respective solutions. Motility was assessed as described in the previous paragraph.

All data is expressed as mean ± standard deviation and considered statistically significant at $p<0.05$, determined by two-tailed Student's t test.

Results and discussions

Glycine causes a dose-dependent reduction in planarian motility

While the control group exhibited a motility count of 12.11±2.44 /min, treatment groups exhibited a significant, dose-dependent reduction in motility, with counts of 8.37±2.67/min (p=0.0182), 6.71±1.19 /min (p=0.0002) and 3±2.11/min (p=0.000007) for the 10μM, 50μM and 100μM concentrations, respectively (fig 1).

Glycine-induced hipomotility is partially reduced by caffeine

The successive administration of glycine (100 μM) and caffeine (50 μM) caused a significant increase in motility counts (9.68±0.72 /min) compared with the glycine-only group (3±2.11 /min, p=0.000004), and a significant decrease compared with control (12.11±2.44 /min, p=0.027) or caffeine only group (19.42±1.42 /min, p=0.0000000017) (fig 2).

* email: ormenisanalina@yahoo.fr

All authors have equal contributions to the study and the publications

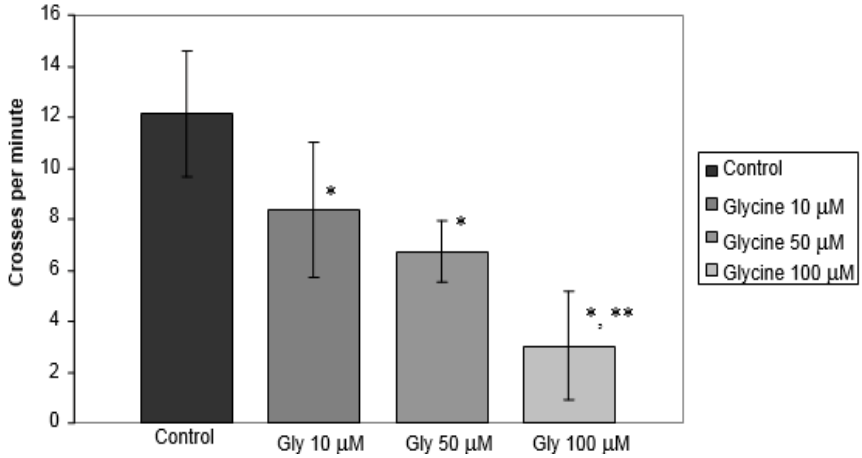

Fig. 1. Glycine causes a dose-dependent reduction in motility. * $p<0.05$ compared with control. ** $p<0.05$ compared with glycine 10 µM and 50 µM. Gly – glycine, followed by the administered dose

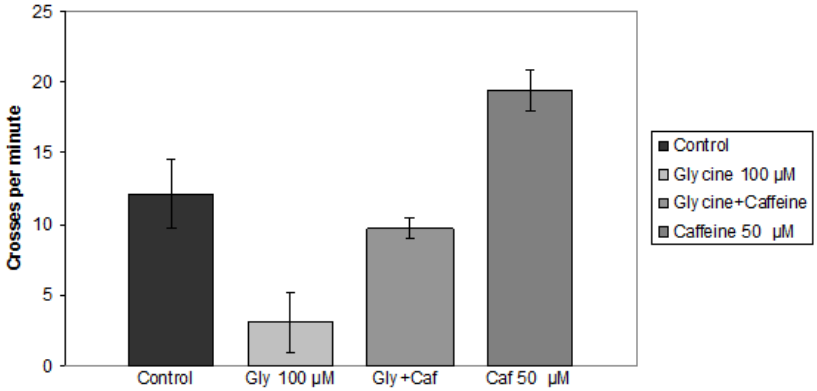

Fig. 2. Caffeine reduces glycine induced hypomotility. $p<0.05$ for glycine+caffeine compared to all other groups. Gly – glycine, Caf – caffeine

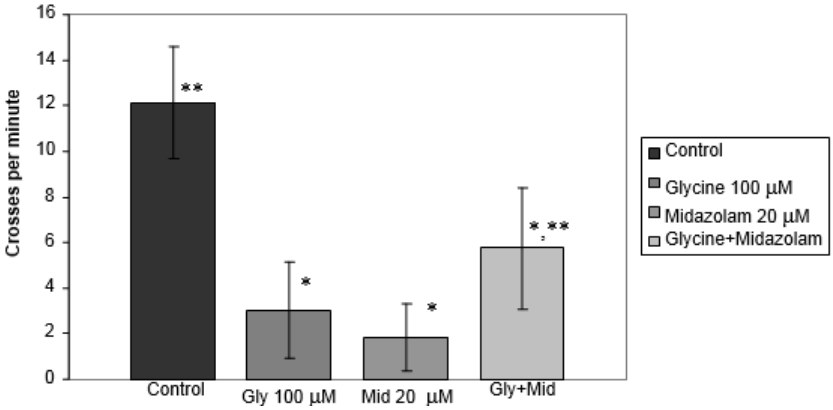

Fig. 3. Midazolam reduces glycine-induced hypomotility. * $p<0.05$ compared with control. ** $p<0.05$ compared with midazolam 20 µM. Gly-glycine, Mid – midazolam

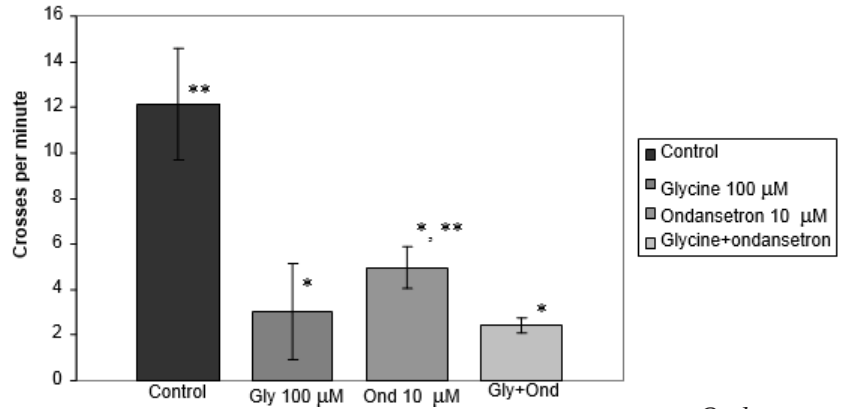

Fig. 4. Ondansetron causes hypomobility. * $p<0.05$ compared with control. ** $p<0.05$ compared with ondansetron 10 µM. Gly – glycine, Ond - ondansetron

Glycine-induced hipomotility is partially reduced by midazolam

Midazolam (20 µM) determined a significant decrease in motility count (1.82±1.46/min, $p=0.000000594$) compared with control. The successive administration of 100 µM glycine and 20 µM midazolam determined a significantly higher motility count (5.74±2.65) compared with midazolam ($p=0.00508$), but not with glycine ($p=0.0557$) (fig 3).

Ondansetron causes hipomotility in planarians

Ondansetron (10 µM) determined a significant decrease in motility counts (4.97±0.91/min, $p=0.00001$) compared with control, but less intense than 100 µM glycine. The successive administration of glycine (100 µM) and ondansetron (10 µM) determined a significant decrease in motility counts (2.42±0.31) compared with control ($p=0.0000002$) and ondansetron only ($p=0.00001$) groups, but not with the glycine only group ($p=0.47$) (fig 4).

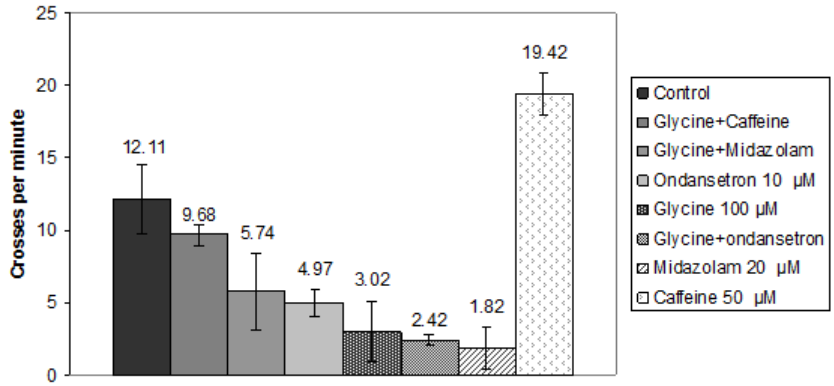

Fig. 5. Comparative effect of all investigated substances on motility. Values above columns represent the average number of crosses per min

Comparison of effects

Overall, the most intense inhibition of motility was caused by Midazolam (20 µM), followed by Glycine (100 µM) + Ondansetron (10 µM), Glycine (100 µM), Ondansetron (10 µM), Glycine (100 µM) + Midazolam (20 µM), and Glycine (100 µM) + Caffeine (50 µM). Caffeine increased motility compared to control (fig 5).

In the present study we have shown that glycine (10, 50 and 100 µM) reduces planarian motility in a dose-dependent manner, which is supported by previous data regarding the presence of glycine in planarians [6], and its effect as an inhibitor of NMDA and AMPA receptors, involved in induced seizure-like activity [7].

The increase in motility after caffeine (50µM) administration to glycine (100 µM) exposed planarians is consistent with the literature, which explains this effect either by glycine receptor antagonism [8], or by adenosine receptor antagonism [9, 10].

Planarian motility was inhibited by midazolam, consistent with its known role as a $GABA_A$ receptor agonist [11]. While the presence of $GABA_A$ receptors in planarians is yet to be proven directly, GABA-ergic transmission is supported by the detection of GABA [6] and glutamic acid decarboxylase [12] in planarians. Our findings regarding the increase in motility after successive administration of glycine and midazolam further supports the presence of both GABA and glycine neurotransmission in planarians and is consistent with the known mutual inhibition of the two systems [13].

Previous studies on rat neurons suggest that ondansetron is an inhibitor of glycinergic transmission [14, 15]. Ondansetron alone had a moderately inhibitory effect on motility, explained in previous studies by its 5-HT antagonist activity [16].

Conclusions

Our results showed no significant increase in motility after successive administration of glycine and ondansetron, compared with glycine alone. Since, to our knowledge, this is the first study involving ondansetron effects in planarians, the difference between our results and previous studies may be attributed to differences between planarian and mammalian nervous systems. However, this is beyond the scope of the present article.

This study brings further evidence regarding the presence of a glycinergic system in planarians, and highlights the potential of planarians as simple model organisms in glycinergic and other neuropharmacological research.

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